THE REPRODUCTION OF LIMACINA RETROVERSA (FLEM.)

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Introduction

The structure of the reproductive system of *Limacina retroversa* and the process of spermatogenesis of this species have been described in a previous paper (Hsiao, 1939). It was shown that very small animals are sexually undifferentiated. The gonads of sexually differentiated but small-sized individuals are protandric and many of them are often without female tissue, that is, "pure male," while those of the bigger animals are essentially hermaphroditic, showing different proportions of male and female tissues. Some individuals function as male, others as female and still others both as male and female at the same time. It is the purpose of this study to examine the relationship between size and sexual phases and the time of the occurrence of the latter so as to determine their sequence in the reproductive history of this form and to compare it with other mollusks whose sexual history is known.

Description of Material

The material was collected by the research vessel "Atlantis" during a series of cruises in the Gulf of Maine between June, 1933 and September, 1934. Specimens collected at the following periods were used:

Date	Designated as
December 2–11, 1933	December specimens
January 9–13, 1934	January specimens
March 21–28, 1934	March specimens
April 17–May 13, 1934	April specimens
May 21–June 2, 1934	May specimens
June 25–July 1, 1934	June specimens
September 17–24, 1934	September specimens

The specimens were caught by vertical hauls made with a 1.5-meter Heligoland larva net drawn from a level near the bottom up to the surface and fixed on board the ship in 2 per cent formalin in sea water.

[1] Contribution No. 206 from the Woods Hole Oceanographic Institution.

Limacina retroversa were later sorted out from the other species and specimens were selected from one to three stations from each cruise so as to make a series covering nearly the whole range of size. Each individual was measured with shell-opening facing the observer under a binocular microscope with a calibrated ocular micrometer. The diameter of the largest whorl of the spiral body of the animal, excluding the protruding margin of the shell-opening (*A–B* in Fig. 1), was measured and used to designate the size of the specimens studied.

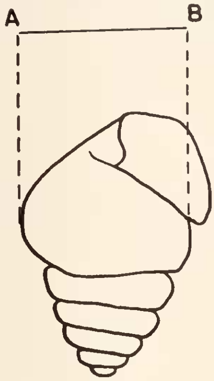

FIG. 1. Camera lucida drawing of the shell of *Limacina retroversa*. × 20. *A B:* maximum diameter of the shell used for comparison of size.

From a study of the size distribution of *Limacina retroversa* from 1933–34 and the movement of the water masses, Redfield (1939) has shown that at least two different populations entered the Gulf of Maine during the period of observation. The first group, called "population A," invaded the Gulf during December, 1933 and January, 1934, and later in the spring a second group, "population B," entered the basin during April and May from the same general direction. The positions of the hydrographic stations from which specimens used in this study were taken are shown in Fig. 2. In this map the solid circles with the abbreviations of the months beside them show the position and time of collection of the material from the population of *Limacina retroversa,* which has been identified as "population A" by Redfield. The positions of the stations providing the material fall within the region where large numbers of "population A" were found (as shown by Redfield's map), that is, in or near the center of density of this population in the different months. On the same map (Fig. 2) the open circles, with the abbreviations of the months in parentheses under them, indicate the stations in April and May from which specimens were selected which represent "population B." From these specimens the reproductive history of the species is worked out. The following table (Table I) shows the number of individuals used from each cruise grouped into size classes with a class interval of 0.3 mm.

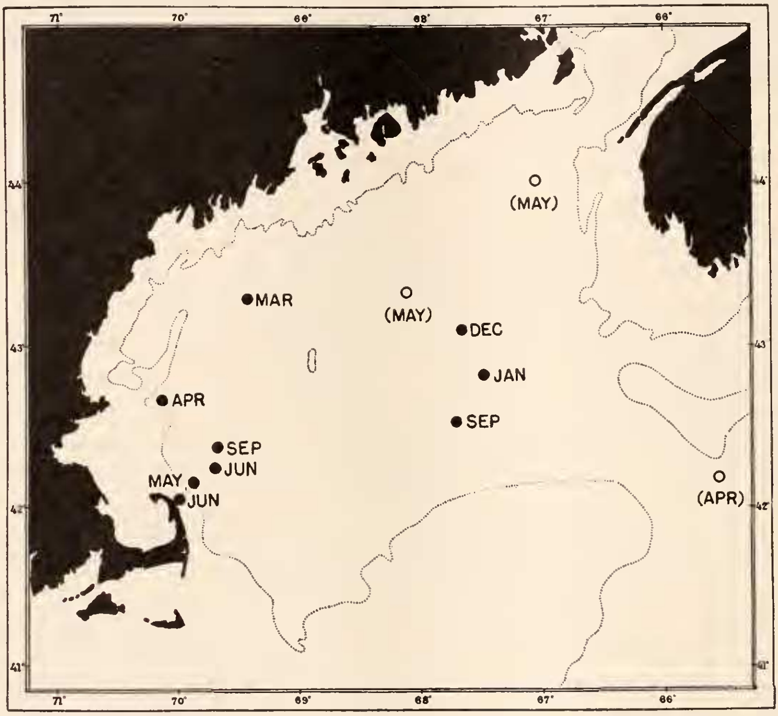

Fig. 2. Map of the Gulf of Maine showing the hydrographic stations from which samples of *Limacina retroversa* are selected for microscopic study.

●: stations for "population A."
○: stations for "population B."

The method employed in making serial sections of these specimens has been described in a previous paper. The following observations are based upon a comparative study of these serial sections.

Relation Between Size and Sex

The relation between size and sex in *Limacina retroversa* has been examined from two different angles: (1) The gonadal types and size, and (2) the relation between functional types and size.

Gonadal Types and Size

The following morphological types are seen among *Limacina.* (*a*) Sexually undifferentiated individuals all of which are small-sized; (*b*) " pure males " which are nearly completely male in constitution and are somewhat larger; and (*c*) hermaphrodites, which have various proportions of male and female tissues in the ovotestis and are generally of large size. The occurrence of the different morphological types of individuals in *Limacina retroversa,* in order of size, is shown in Table II.

Sexually Undifferentiated Individuals.—Of all the *Limacina retroversa* sectioned the largest individual which has a gonad still in a sexually undifferentiated condition measures 0.85 mm. in diameter. Individuals larger than this size all show definite gametogenesis. All specimens with a diameter less than 0.6 mm. are sexually undifferentiated. Hence, the process of sexual differentiation must commence during the period when the animal is between 0.6 mm. and 0.85 mm. in diameter. This is brought out more clearly when the percentage of sexually undifferentiated individuals is plotted against size as shown in Fig. 3.

TABLE I

Table showing the number of individuals from each period of collection which were used in this study. The individuals are separated according to size into groups with a class interval of 0.3 mm.

Month of cruise	Size groups in mm.								Total number obtained during month
	0 to 0.3 mm.	0.3 to 0.6 mm.	0.6 to 0.9 mm.	0.9 to 1.2 mm.	1.2 to 1.5 mm.	1.5 to 1.8 mm.	1.8 to 2.1 mm.	2.1 to 2.5 mm.	
Dec.	3	13	9	8					33
Jan.		13	12	4	3				32
Mar.		4	4	7	10	1			26
Apr.			5	9	5	6	1		26
May	4	11	7	6	3	6	2	1	40
June	4	3	6	2	6	5	2		28
Sept.	1	6	8	7	4	3			29

"Pure Males."—These are animals with gonads which contain little or no noticeable amount of ovarian tissue. They correspond to Coe's " true males " or Orton's " pure males." These individuals are confined to comparatively small-sized groups. Table II, column 8, shows the sudden appearance and the steady but more gradual decrease of " pure males " among animals arranged in the order of increasing size. Among larger animals this type of individual gives place to those which have

various proportions of feminine reproductive cells in the gonads. These are the hermaphrodites described in the next paragraph. On the average, about 22 per cent of the specimens less than 1.2 mm. in diameter belong to the " pure male " class. Many of these males are functional at the time of fixation as shown by the presence of mature spermatozoa in the gonoduct.

Animals with Various Proportions of Male and Female Tissues in the Gonads.—In addition to the sexually undifferentiated animals and the " pure males " a small number of animals less than 1 mm. in diameter

TABLE II

Proportion of different morphological types of *Limacina retroversa* separated into groups without regard to the time of collection. Animals smaller than 1.2 mm. in diameter are separated into 0.1 mm. classes, those larger than 1.2 mm., into 0.3 mm. classes.

1	2	3	4	5	6	7	8	9	10
Size in mm.	Total number	Undifferentiated	Pure male	>50% male	<50% male	Percentage undifferentiated	Percentage pure male	Percentage of >50% male	Percentage of <50% male
0.4	3	3	0	0	0	100	0	0	0
0.5	3	3	0	0	0	100	0	0	0
0.6	13	8	3	2	0	62	23	16	0
0.7	15	5	7	3	0	33	47	20	0
0.8	24	3	8	13	0	13	33	54	0
0.9	12	0	3	7	2	0	25	58	17
1.0	14	0	2	8	4	0	14	57	28
1.1	15	0	2	10	3	0	13	67	20
1.2 to 1.49	44	0	0	34	10	0	0	77	23
1.5 to 1.79	34	0	0	26	8	0	0	77	23
1.8 to 2.09	19	0	0	11	8	0	0	58	42
2.1 to 2.39	4	0	0	2	2	0	0	50	50
2.4 to 2.7	2	0	0	1	1	0	0	50	50

may have gonads containing various proportions of male and female tissues at the same time. Of all the animals greater than 1.1 mm. in diameter no undifferentiated or " pure males " were found. They all contain spermary and ovarian tissues in the same gonad. Following the criteria described in a previous paper these hermaphroditic animals can be classified as hermaphroditic males or hermaphroditic females according to whether they have more than 50 per cent or less than 50 per cent of their gonadal tissues consisting of male reproductive elements. The proportion of these two kinds of individuals in the different size groups is shown in Table II. Column 5 shows the number of individuals with more than 50 per cent of their gonadal tissue made up of masculine elements, excluding those which are already listed in column 4 as "pure males." It shows the number of hermaphroditic males only. The percentages of hermaphroditic males of this class are given in column 9. Column 10 shows the percentages of hermaphroditic females based on the numbers shown in column 6. A comparison of columns 9 and 10 will show that the minimal size of the hermaphroditic females is much greater than that of the hermaphroditic males. This occurrence of hermaphroditic males as well as "pure males" in smaller-sized animals when compared with the hermaphroditic females indicates that the male reproductive tissue is developed when the animals are smaller than is the case with the female cells of reproduction.

Owing to the small number of individuals in each size group among the larger animals the data in the lower half of Table II have been rearranged so that the class interval is three times as large as in the upper half. It will be seen that among animals smaller than 1.2 mm. the proportion of hermaphroditic males increases steadily while among those larger than this size the percentages decrease from 77 to 50. On the other hand, there is only a small proportion of hermaphroditic females among the smaller animals, but among the larger ones the percentage increases from 23 to 50. Approximately speaking, before the animals reach 1 mm. in diameter the proportion of males increases very rapidly, indicating an early proliferation of the male reproductive cells giving rise to the types of males whose gonads are predominantly masculine in appearance. After the animals grow to a size greater than 1.5 mm. in diameter the female reproductive cells overtake the male in development, thus bringing about a decrease in the proportion of hermaphroditic males after the 1.8 mm. size group and an increase in the predominantly feminine type. The decrease in the proportion of animals which are predominantly masculine continues until the animals are about 2 mm. in diameter, from which stage onward the two sexual types are equally numerous (Fig. 3).

The Relation between Functional Types and Size

In addition to the above morphological types, we can analyse our material into functional types. From the structure and the place of occurrence in the body of mature germ cells each *Limacina's* state of reproductive activity at the time of fixation can be deduced. Thus, when mature spermatozoa are seen in the gonoduct, or at the base of the penis, the individual is obviously functioning as a male and can be classified as an *active male*. When mature ova are found in the ovotestis the individual can be considered as a *mature female,* while the presence of ripe eggs in the gonoduct indicates that the individual is a *spawning female.* The simultaneous presence of mature spermatozoa in the gonoduct and ripe ova in or out of the gonad is an indication that the individual is a *functional hermaphrodite,* to use Coe's terminology. The occurrence of these functional types is shown in Table III.

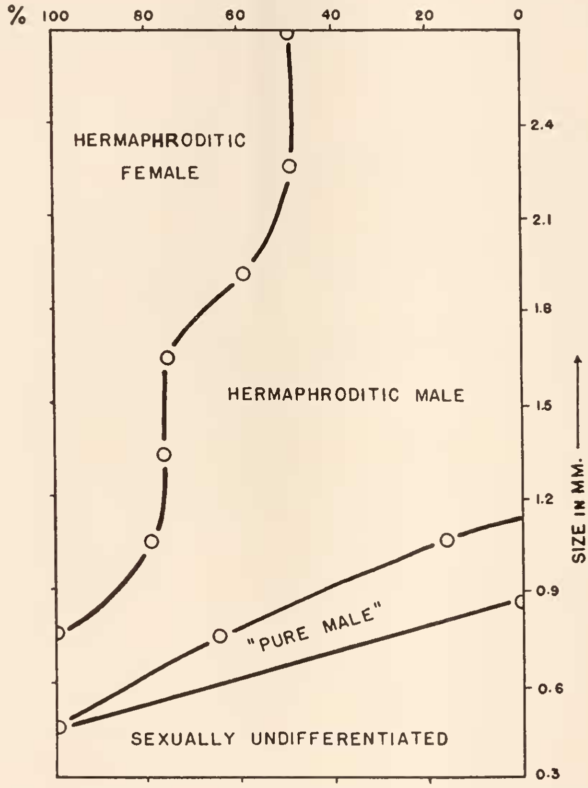

Fig. 3. Diagram showing the relation between size and various morphological types of gonads of *Limacina retroversa* (expressed in percentages of the total of each size group).

It has been observed that some individuals as small as 0.7 mm. in diameter may function as males. But the majority of specimens actively functioning as males only are between 1.2 mm. and 1.8 mm. in diameter (column 4). In larger-sized groups their place is taken by the females and individuals functioning both as male and female simultaneously.

The proportion of mature females with ripe eggs in the gonad, on the contrary, increases with the increase of size. The class with the largest number of mature females, as shown in Table III, is the 2.1 to 2.4 mm. group. The smallest *Limacina* with mature ova is more than 1 mm. in diameter. At the time of fixation many mature females were in the act of spawning, as evidenced by the presence of ripe ova at various points along the gonoduct. Roughly speaking, about half of the mature females in each size group were in the act of spawning when fixed.

The proportion of the third functional type, functional hermaphrodites, in each size group is shown in column 6 of Table III. The chief occurrence of this type of animal is among the larger-sized specimens. It will be seen from Table III that although both specimens of the 2.4–2.7 mm. size group are functional hermaphrodites, the number of individuals used is so small that no great importance can be attached to the

TABLE III

Proportion of different functional types of *Limacina retroversa* expressed as percentage of each size group irrespective of the time of collection. Class interval: 0.3 mm.

1	2	3	4	5	6	7
Size groups (mm.)	Total number	Undifferentiated	Active male	Mature female	Functional hermaphrodite	Without signs of activity
		per cent	*per cent*	*per cent*	*per cent*	*per cent*
0.3–0.6	6	100	0	0	0	
0.6–0.9	52	30	17	0	0	53
0.9–1.2	41	0	39	15	5	41
1.2–1.5	44	0	48	9	14	29
1.5–1.8	43	0	47	18	21	14
1.8–2.1	19	0	5	42	10	43
2.1–2.4	4	0	0	50	50	0
2.4–2.7	2	0	0	0	100	0

deduced percentage of this type of animal. Until more large specimens are studied it seems better to leave this group out in our examination of the relation between the functional types and size. In the 2.1–2.4 mm. size group mature females and functional hermaphrodites are equally numerous.

When the gonads of these functional hermaphrodites are examined and classified in terms of the proportion of masculine and feminine tissue in them the following relationship is seen.

Type of gonad:	Percentage of specimens
With more than 75% male tissue	57%
With 50%–75% male tissue	33%
With 25%–50% male tissue	10%
With less than 25% male tissue	0%

Most functional hermaphrodites have more than 75 per cent of the gonadal tissue made of male reproductive cells, one-third of them have a 50 per cent to 75 per cent male type of gonad, while no *Limacina* with less than 25 per cent male tissue in the gonad, that is, no very pronounced female type of individual, functions as hermaphrodite. Since the gonad of this species of pteropod changes from predominantly male to predominantly female type as the animals grow (see Fig. 3), this relation between the proportion of functional hermaphrodites and the amount of masculine tissue in the gonad can be interpreted to mean that animals possessing " more than 75 per cent male " type of gonad later have their female germ cells developed while the male phase of reproduction is still functioning, thus giving rise to a large proportion of hermaphrodites functioning as male and female at the same time. By the time the gonads change over to the predominantly feminine condition the male phase of sexual activity also disappears and hence few or no functional hermaphrodites are seen among individuals with a pronounced feminine type of gonad.

Figure 3 summarizes the facts about the relation of size to sexuality. Sexual differentiation commences during the time when the animals are between 0.6 and 0.9 mm. in diameter. The male germ cells proliferate in smaller-sized individuals giving rise to the " pure males " and hermaphroditic males before the animal reaches 1.2 mm. in diameter. The proportion of " pure males " decreases with increase in size. As the size increases further, the proportion of hermaphroditic males decreases, while that of the hermaphroditic females becomes greater and greater until the two sexual types are equal in number among animals greater than 2 mm. in diameter.

Functionally the situation is summarized in Fig. 4. The male phase starts among smaller *Limacina* than the functional female and hermaphroditic phases. The highest proportion of actively functioning males is found among animals 1.2 to 1.8 mm. in diameter. The large proportion of mature females and functional hermaphrodites occurs among larger animals replacing the male phase in those size groups. Females become mature after they have attained a diameter greater than 1 mm. It is probable that mature females and functional hermaphrodites are equally numerous among animals greater than 2 mm. in diameter. The percentage of each of these three functional types among the different size groups is shown in Fig. 4.

Seasonal Changes in the Reproductive Cycle

When the condition of the reproductive system of individuals collected at different times of the year is examined comparatively it is found that, as a general rule, in winter individuals are small protandric males and that later hermaphrodites and functional males and females appear. We shall first take up the morphological types of sexual individuals and the question of the proportion of these sexual types in different months, then the appearance of different sexual activities at various times of the year and lastly the question of the reversal of sex predominance. It has

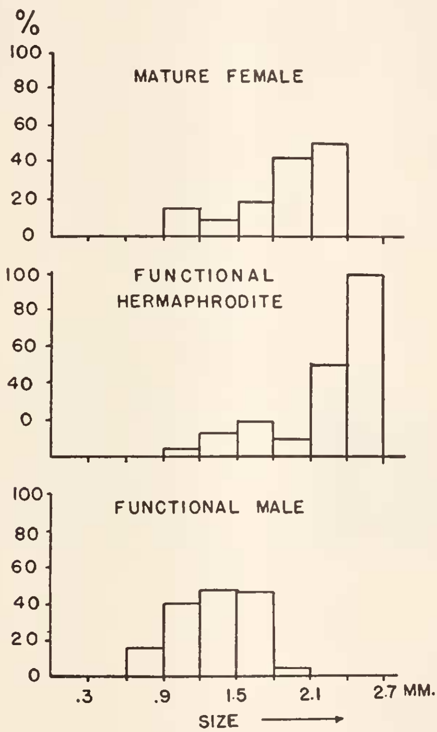

FIG. 4. Distribution of the three functional types of *Limacina retroversa* among various size groups. Functional males occur among smaller-sized animals than functional hermaphrodites and mature females.

been shown by Redfield (1939) that "population A" can be clearly differentiated from "population B" from December up to May and after that time there is a good deal of mixing of populations with "population B" predominating over the descendants and remnants of "population A" and new recruits into the Gulf. In the following description data from December to May refer to "population A" and those after May, to the mixed populations.

Change in Gonad Structure

Young sexually undifferentiated individuals were found among the specimens taken in each month except March and April. Figure 5 shows the percentage of the individuals taken each month which are sexually indefinite. During the winter months of December and January "population A," which entered the Gulf then, contained 14–16 per cent of sexually undifferentiated animals. In March and April these animals drifted to the western portion of the Gulf and grew larger, as shown by Redfield, and there remained no sexually undifferentiated individuals. After May, a portion of the animals examined, 19–20 per cent, were

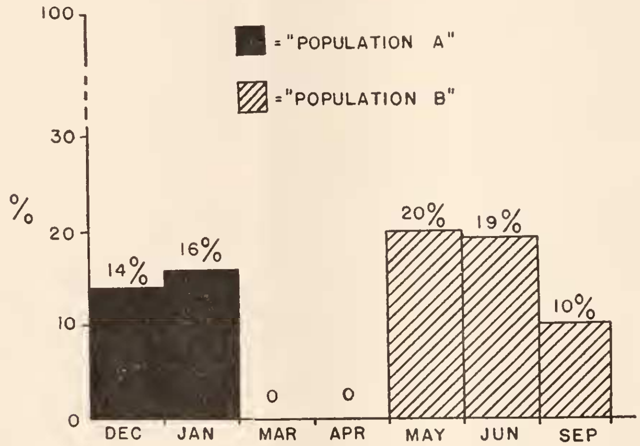

FIG. 5. Histogram showing the percentage of sexually undifferentiated individuals in each month. " Population A " solid black, " population B "shaded with straight lines. About 15 per cent of the material caught (in the winter) from " population A " and 20 per cent from " population B " are sexually undifferentiated. Absence of immature material in March and April indicates that members of " population A " are all differentiated by March.

again sexually indefinite. This coincides with the entrance into the Gulf of a new population in the east and the spawning of " population A " in the west portion of the basin. As far as " population A " is concerned, sexual differentiation is completed by the month of March and all the individuals of this population were either developing in the direction of males or hermaphrodites.

Animals which were sexually differentiated have been divided into male or female type according to whether they had more or less than 50 per cent of their gonadal tissue made of male reproductive cells. The proportions of these male and female types among sexually differentiated *Limacina* collected each month are shown in Fig. 6. In this figure the material before May refers to "population A" and that after May to the mixed populations, with "population B" as the predominating constituent. It will be seen that in December and January nearly all the sexually differentiated *Limacina* were predominantly male. After these months the proportion of predominantly male individuals became reduced: in April they constituted only 40 per cent, while in May the two sexual types were equally numerous. After May the proportion of predominantly male animals became more numerous again among the mixed populations.

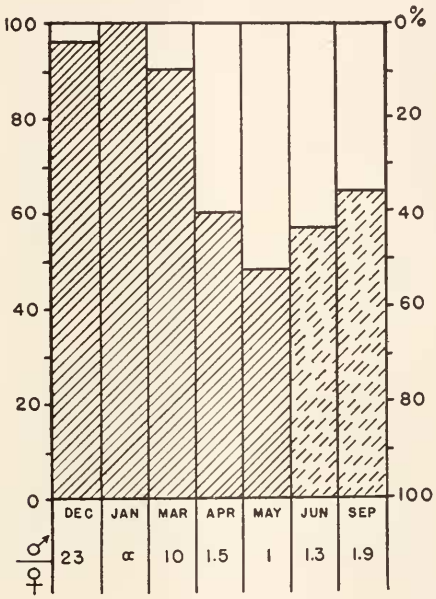

FIG. 6. Proportions of hermaphroditic males and hermaphroditic females among sexually differentiated *Limacina retroversa* in each month. Unshaded area represents hermaphroditic females, shaded areas hermaphroditic males. Area shaded with continuous lines refers to "population A," that with broken line, to "population B." ♂/♀ refers to the ratio of sex predominance in each month.

When the change in the percentage of the two sexual types among sexually differentiated *Limacina* from month to month as shown in Fig. 6 is calculated into ratios of sex predominance (the number of predominantly male individuals over those predominantly feminine) a change from a high value to unity is seen. These values are appended at the bottom of Fig. 6. It will be seen that in December and January this value is very high, but in May it is equal to one, while among the mixed populations the value is again in favor of the male type in the later part of the year.

In Fig. 7 are shown the proportions of "pure males," animals with little or no feminine tissue in the gonad, and "less than 25 per cent" male (very pronounced feminine) types among the sexually differentiated individuals in each month. About 45–55 per cent of sexually differentiated *Limacina* in December and January were "pure males," the rest being hermaphroditic males. In March the proportion of "pure males" was reduced greatly and from April onward this type of individual was not seen any more. On the other hand, pronounced feminine individuals with less than 25 per cent of the gonadal tissue made of male germ cells were not seen before May. It will also be noticed that

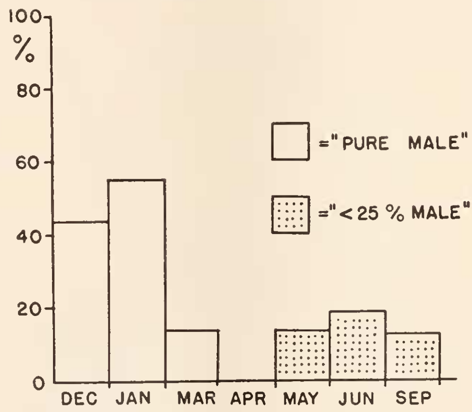

FIG. 7. Monthly appearance of "pure males" and hermaphroditic females with less than 25 per cent of their gonadal tissue consisting of male germ cells. Histograms before April refer to "population A" and those after April to "population B" with mixed populations. "Pure males" represented by unshaded areas; "less than 25 per cent male" type of hermaphroditic females represented by shaded areas.

the occurrence of this type of extreme female in the later part of the year has been always low in comparison to the other types.

Change in Reproductive Activity

The reproductive activities of *Limacina retroversa* show a parallelism with the morphological changes associated with the advance of time. During December and January when "pure" and hermaphroditic males constituted nearly all the groups of sexually differentiated individuals no hermaphroditic females with mature ova in the gonad were seen in the specimens examined, while spermatozoa, on the other hand, were evidenced as being liberated by the fact that mature male germ cells were found in different parts of the gonoduct. It is in March, however, that individuals with mature ova in their ovotestes began to appear. This type of individual continued to increase in proportion until May. In May animals had been collected and fixed during their spawning activity. Later in the year all the different types of functional individual were seen. The occurrence of these types of functional individual in the various months is shown in Table IV.

Table IV

Distribution of functional types of *Limacina retroversa*, expressed in percentages, in different months.

1	2	3	4	5	6
Month	Number studied	Active males	Mature females	Spawning females	Functional hermaphrodites
		per cent	*per cent*	*per cent*	*per cent*
Dec.	24	63	0	0	0
Jan.	26	58	0	0	0
Mar.	22	55	5	0	0
Apr.	25	32	20	0	8
May	23	17	43	30	26
June	21	19	38	19	19
Sept.	26	19	27	12	35

It will be seen from column 3 of this table that individuals ready to liberate spermatozoa are seen throughout the year, but that the proportion is higher in the earlier part of the year than in the later and that the reduction of the number of active males is accompanied by an increase in the number of individuals functioning as females (columns 4 and 5) and those acting as hermaphrodites (column 6) after April. A comparison of columns 4 and 5 will show that spawning is found after the mature females were observed in March. It has been found in the section on the relation between size and sexuality that about half of all the mature females examined were fixed during the act of spawning. From column 5 of Table IV it is seen that in May about three-quarters of the mature females were fixed in this stage, while in June the proportion of spawning females amounted to half of the matured ones, and in September, less than half. This indicates a higher spawning activity in May than in the other months. The fact that no spawning individuals were seen among the mature females in April does not exclude the possibility that egg-spawning took place in this month but that the individuals did not happen to be fixed with eggs in the gonoduct and selected for examination in this study. We may consider spawning to start between April and May and continue to the fall with May as the most important period for breeding.

From the above examination of the reproductive system of *Limacina* in terms of time it appears that among " population A," which entered the Gulf of Maine first, all the individuals were sexually differentiated by the beginning of March. In winter, a great majority, if not all, of them were " pure males." The number of such " pure males " decreased in March and they were not seen in April, giving place to individuals with a lesser degree of masculinity, to functional hermaphrodites and eventually to hermaphroditic females with very little masculine tissue in their gonads. The proportion of male tissue in comparison with the female in the gonad decreased as the animals grew. By April 40 per cent of the specimens collected had the feminine tissue so well developed as to warrant their being classified as hermaphroditic females. Animals with " less than 25 per cent male " gonads occurred after the " pure males " had disappeared from the collections. In May the two sexual types were equal in number. After May the bulk of the species in the Gulf was derived from the recently arrived " population B " which mixed with the descendants and remnants of " population A " and other new recruits. Among the mixed population sexually undifferentiated individuals were seen again and all the structural types were present.

The functional phases of reproduction are summarized in Fig. 8. More than half of the protandric males started to function in winter and the male phase continued into spring and summer as it became gradually replaced by the female and functional hermaphroditic phases of reproduction. Females began to attain maturation in March and the proportion of mature females increased rapidly in April and May. Spawning of ova commenced between April and May, being highest in the latter month. The largest proportion of functional individuals, including the male, female, and hermaphroditic types, is seen in May when half of them were spawners of eggs and the other half were males and hermaphrodites in function. These individuals were seen in the months from June to September also. It appears that May is the most important period for spawning though the reproductive activity was carried on into the autumn.

Change in Sex Predominance

A change in the value of the ratio of the sexes during growth is generally used as one of the indications of a change of sex. As the term " sex ratio " is not very appropriate for monoecious forms where both male and female reproductive tissues are present in the gonad, it seems better to speak of sex predominance and ratio between male and female predominance. The decrease in the value of this ratio from small to large-sized animals may be due, in general, to any one of the following causes: (*a*) Sexual dimorphism such that the males are habitually smaller than the females, (*b*) differential mortality, that is, more males than females die as the animals grow, and (*c*) sex reversal or an actual change in the condition of the reproductive system as the animals grow. There is no evidence from our material either to show that there

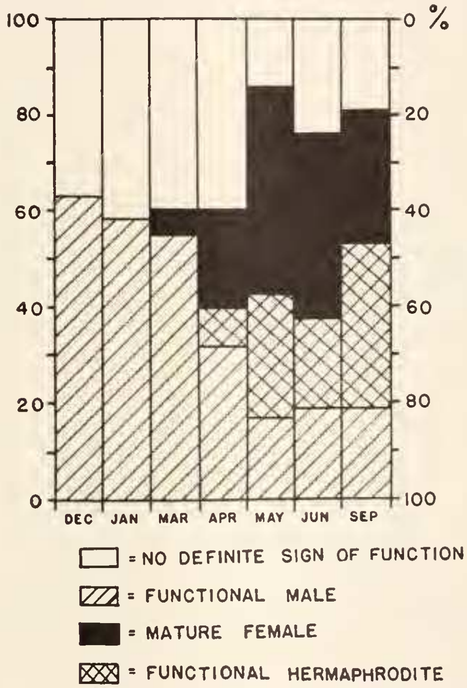

FIG. 8. Monthly distribution of the three functional types of *Limacina retroversa.*

is a sexual dimorphism or to indicate a differential mortality which may be interpreted as the cause of the appearance of a higher proportion of males among smaller-sized *Limacina* and a greater number of females among larger ones. The third alternative, that a change in the predominant sexuality takes place as the animals grow, however, has much evidence in its favor. In the first place, it has been shown above that there is a parallelism between the changes in the ratio of sex predominance with reference to size and that with reference to the advance of the season: male types dominate among smaller-sized *Limacina* and during the winter months, and this excess of males decreases as the animals become larger and also as the season advances, and eventually the two types become equal among still larger-sized specimens and at the height of the breeding season in May. This parallelism indicates a change in the sexuality, both in structure and function, of the individuals as they grow.

In the second place, it has already been shown in a previous paper that there is no clear line of demarcation between male and female among *Limacina,* but that hermaphroditic male and female merge into each other and that a continuous series can be made out. As the size of the animals increases, a greater and greater portion of the gonad appears to contain female tissue. "Pure males" are confined to small-sized animals and in the winter months. The continuous change in the proportion of male and female reproductive tissues in the gonad culminating in many functional hermaphrodites and females with less than 25 per cent of the tissue of their sex glands made of masculine elements argues in favor of a reversal of sex predominance in this species. Furthermore, there is no "pure female" among *Limacina:* all the hermaphroditic females are large and apparently derived from male types.

Thirdly, a comparison of the size distribution of the different sexual types in the various months also shows that there is a change in sex predominance. In Fig. 9 the proportions of (*a*) sexually undifferentiated young, (*b*) individuals which are predominantly masculine and (*c*) those which are predominantly feminine, have been indicated in the histograms prepared by Redfield showing the distribution of size among all the specimens taken during each cruise. It shows the earlier occurrence of protandric males and later appearance, in March and April, of female types. The appearance of the female type among larger size groups suggests that they are transformed from the protandric individuals rather than from the very small undifferentiated ones.

From these morphological male and female types of *Limacina* functional hermaphrodites have also been indicated in the same figure (Fig. 9). These functional hermaphrodites increase from a very small proportion in April to a maximum in May and then decline in June. This change in the relative proportion of this type of individual supports the idea mentioned above that protandric males develop ovarian tissue while they are still functioning as males and eventually become functional females.

Finally, if the modal class of each month's collection is taken as representative of the total catch, the phenomenon of change of sex predominance can be brought out when the different modal size groups are compared. Analysis of the size frequency distribution showed that

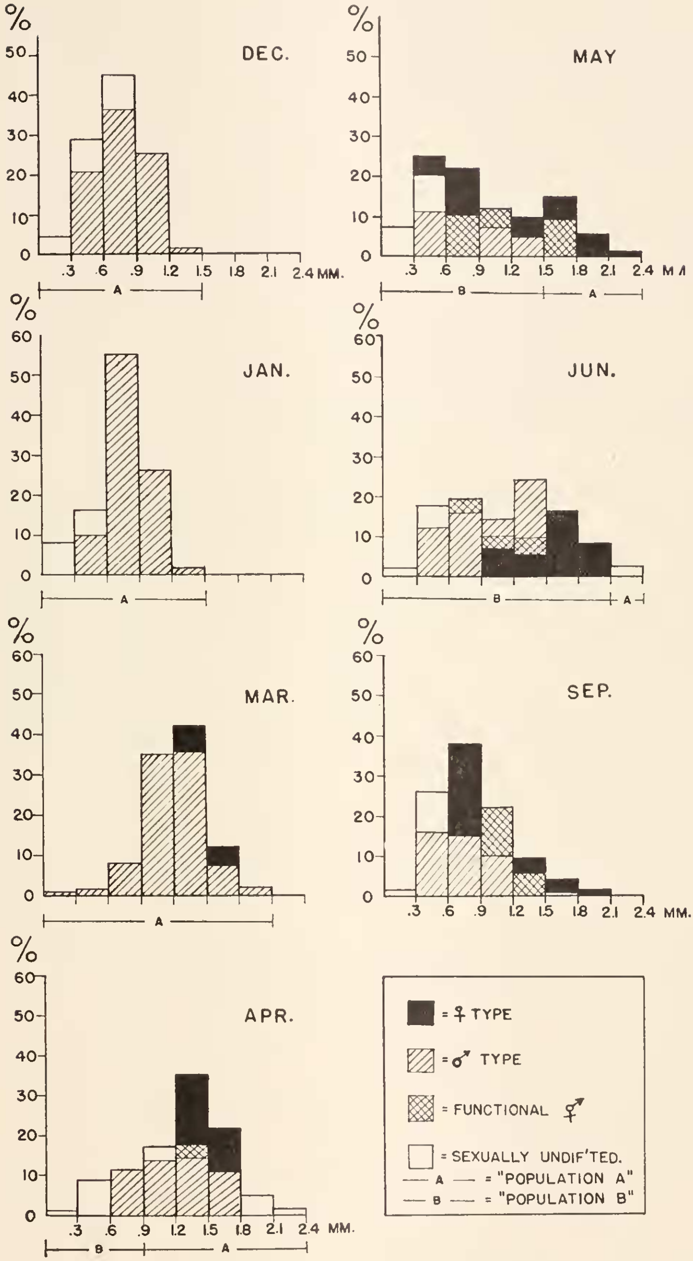

FIG. 9. Distribution of different types of *Limacina retroversa* among various size groups in each month. The proportion of each sexual type is added to the histogram showing size distribution prepared by Redfield (1939).

there is a single mode from December to April (Redfield, 1939). This modal class shifts from smaller to larger size during this period, showing a general growth of the members of the population. The sexual condition of the gonads of the modal class shows that not only growth, but sexual change has also taken place. In the modal class of the December material all the differentiated individuals are males and over 60 per cent of them have more than three-quarters of their gonadal tissues composed of male reproductive cells. Half of the males are ready to liberate spermatozoa. In January all the individuals of the modal class are predominantly male and 65 per cent of them are ready to discharge the product of their sex glands, thus showing an increase in the proportion of sexual differentiation with growth and an increase in the number of individuals capable of inseminating others. But it is in March that 11 per cent of the modal class are seen to have more female tissue in their gonads; the rest are still all predominantly male, half of which are ready to discharge or are discharging spermatozoa. The proportion of females increases to one-half of the modal class in April and some of the individuals begin to function both as male and female simultaneously. Of the two modal classes in May, the larger one shows, in the condition of the reproductive system, a continuation of the general changes of the previous months. For there is a further reduction of the male type and an increase of the functional hermaphrodites and actively functioning females. In this month, when the egg-laying individuals are added together, that is, spawning females plus functional hermaphrodites, they make up the whole of the modal class (see the right half of the histogram for May in Fig. 9). Thus if the modal class found by Redfield is taken as representative of the material collected, observations on the sexual condition of these groups give a consistent story of the change of sex predominance and function.

Relation between Sexual Phases and Distribution

When the sexual condition of populations A and B are compared several interesting differences are seen. The first characteristic of the sexual phases of " population B " in contrast to " population A " is that sexual differentiation seems to commence earlier with reference to size. In " population A " individuals as large as 0.85 mm. in diameter have been seen as undifferentiated sexually, while the largest undifferentiated individual of " population B " is only 0.6 mm. in diameter. In addition to the earlier differentiation of the gonads, a second difference is seen in the manner of differentiation between the two populations. Instead of the male elements in the gonads always taking the lead in development so as to bring about protandry, some individuals among " population B " have both types of sexual cells developed at nearly the same time in addition to protandry, so that proterogyny and hermaphroditism appear. In view of this type of development, small-sized mature hermaphroditic females and functional hermaphrodites may also be interpreted as derived directly from young undifferentiated individuals with the protandric stage omitted. In " population A " such a situation is not seen. The third point of difference is that the development of the female elements of the gonads of individuals in " population B " is also earlier. This can be seen when the minima of the size of the individuals which achieved maturation of ova in the two populations are compared. The minimal size of *Limacina* with mature ova is 0.8 mm. in diameter in April and 1 mm. in May among members of " population B," while among " population A " the minimal size of mature females is 1.3 mm. in April and 1.67 mm. in May. It is possible that these small mature females in " population B " did not pass through a protandric stage of noticeable length, though other specimens of the same population have a distinct young male phase preceding the female. In " population A " the smallest functional hermaphrodite is 1.5 mm. in diameter in April and 1.6 mm. in May, while during the same months functional hermaphrodites as small as 1.1 mm. in diameter are found among " population B."

In Fig. 9 the right portion of the May histogram which has been attributed to " population A " by Redfield is very similar to that of April in the distribution of sexual types. In both cases hermaphroditic females occupy the large size groups with functional hermaphrodites between them and the small-sized, predominantly male individuals. On the other hand, the left portion which represents " population B," has a size distribution comparable to those of the winter polygons but the sexual phases are quite different from them. Here the hermaphroditic females and functional hermaphrodites appear among protandric males in small size groups, which in the previous months only contain protandric and " pure " males.

All these differences in the sexual condition of the two populations seem to point convergingly to one underlying factor, namely, that during the warmer part of the year sexual differentiation takes place sooner than in the colder time and the sexual phases may be telescoped together when differentiation is speeded up in the summer. This is in line with the previous observations made on *Ostrea* by authors on both sides of the Atlantic (Coe, 1932, 1936; Needler, 1932; Orton, 1926; Spärck, 1925). However, this does not exclude the possibility that there is a difference in the origin of "populations A and B" which may account for some of the observed differences in sexual development.

Comparison of the Reproduction of Limacina retroversa with Other Mollusks

As the reproductive history of no other Opisthobranchiata has been described, it is not possible to compare the reproduction of *Limacina retroversa* with that of the members of the same genus or family or even order. Only in a general way can the sexual sequence of this form be compared with that found among other mollusks.

In the winter months *Limacina* which entered the Gulf of Maine are of small size and sexually undifferentiated. As they grow this initial phase is followed by protandry in which nearly all the individuals are predominantly male. The protandric phase is succeeded by a hermaphroditic phase during which the animals transform from a more masculine to a more feminine condition. In the final phase the individuals are predominantly female in structure and generally function as hermaphrodites, liberating both ova and spermatozoa, or as females only. This type of sexual transformation conforms in a general way with that found among gastropods. The sequence approximates most closely to that of the prosobranchiate gastropods such as *Valvata tricarinata* worked out by Furrow (1935) and *Patella vulgata* by Orton (1928). But the time required for the transformation of sex is different. Orton observed that the male *Patella* transformed into female at the age of one year, while Furrow found that in *Valvata* the different sexually functional phases followed each other very closely and the protandric phase was very short. However, in *Limacina retroversa* the change in sex predominance seems to take place during a period of two or three months. For instance, in one population which entered the Gulf of Maine in December and January, the protandric males were transformed into females from March to May. In the warmer part of the year this transformation required a shorter time for its completion.

Summary

1. In the winter months sexual differentiation commences when the animals are between 0.6 and 0.85 mm. in diameter. In the summer sexual differentiation starts when the animals are smaller.

2. The early proliferation of the male reproductive cells leads to a protandric phase. The "pure males" give place to hermaphroditic males and later to hermaphroditic females as the size of the animals becomes greater than 1 mm. in diameter. After the specimens are 1.5

mm. in diameter the female reproductive cells overtake the male in development, bringing about a decrease in the proportion of hermaphroditic males and an increase of the opposite sexual type. This change continues until the two types are equal, when the animals are 2 mm. or more in diameter.

3. Functionally the male phase starts among smaller *Limacina* than do the functional female and hermaphroditic phases. The highest proportion of actively functioning males is found among animals 1.2 to 1.8 mm. in diameter. Among larger animals the functional hermaphroditic and functional female phases replace the male phase. Hermaphroditic females begin the functional phase after they are greater than 1 mm. in diameter.

4. Members of a population which entered the Gulf of Maine in the winter were all sexually differentiated by the beginning of March. Nearly all of these individuals developed into protandric males, about half of which were "pure males." The number of such "pure males" decreased in March and disappeared in April, being replaced by animals with greater predominance of feminine tissue in the gonads. By April about 40 per cent of the specimens were hermaphroditic females and in May the two sexual types were equal.

5. The male functional phase began in winter and continued into spring and summer. Hermaphroditic females matured in March and their proportion increased in April and May. Spawning of ova commenced by the end of April. The largest proportion of functional individuals of all types was seen in May.

6. Reasons are advanced for the belief that the change in sexual constitution of the populations is due to a reversal of sex predominance of the individuals.

7. The sequence of the sexual phases of *Limacina retroversa* agrees in a general way with those found for other members of the gastropod group of mollusks, particularly that of *Valvata*.

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